The assessment of oral squamous cell carcinoma

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CHAPTER 9

General discussion and future perspectives

GENERAL DISCUSSION

Current evidence and consensus for neck staging in early stage (cT1-2N0) OSCC using the SLNB procedure

A major problem in early stage (cT1-2N0) oral squamous cell carcinomas (OSCC) is that eventually 23-37% of these patients are diagnosed with occult metastases [1]. Conventionally, neck staging was done with an elective neck dissection (END) or a watchful waiting strategy in these patients [2]. In 1994, Weiss et al. proposed that if the risk of occult metastasis was more than 20%, an END was recommended over a watchful waiting [2,3]. That 20% risk was mainly based on T status and anatomical location [4,5]. After multiple studies showed a relationship between tumour infiltration depth and the risk of occult metastasis, tumour infiltration depth with a 4 mm cut-off was added for neck strategy selection [4]. In 2015 a prospective randomized controlled trial analysed 255 watchful waiting and 245 elective neck dissection cases and found a higher five year overall survival and disease free survival for the END (80% and 70% respectively) compared to watchful waiting (68% and 46% respectively) [6] and concluded that END was superior over watchful waiting in early stage OSCC. A major consequence of subjecting all patients to an END is that in the majority (~75%) of the early stage OSCC cases no neck metastasis will be present, while patients are at risk for developing surgery induced morbidities such as shoulder dysfunction [2].

A new era with less invasive neck staging in early stage OSCC started with the introduction of the sentinel lymph node biopsy (SLNB) procedure. Although the SLNB is still an invasive procedure, it is minor surgery compared to the END, which is reflected by a smaller incision (48 mm versus 92 mm [7]), shorter hospital stay (1 versus 3 days [8]) and lower complication rates. In a study with 33 patients staged by SLNB and 29 by END, all 15 postoperative complications appeared in the END group (bleeding n = 5, nerve injury = 8, infection n = 1 and tracheotomy n = 1) and no complications were seen in the SLNB group [7]. Two other studies reported better postoperative shoulder functions and also a smaller scar: 84.2 mm and 73.9 mm for the SLNB group, compared to a scar of 183.3 mm and 171.5 mm in the END group [9,10]. Although the less invasive character is important, correct staging of the neck is the main reason for the use of the SLNB procedure. A meta-analysis using 66 studies in 2017 reported a high pooled sensitivity of 87% and pooled negative predictive value (NPV) of 94% for the SLNB procedure in detecting occult metastasis in early stage OSCC [1]. Limitation of that meta-analysis was the heterogeneity in study protocols with differences in experience of the surgeons, reference standard for the negative SLNB (clinical follow-up or END), preoperative imaging procedures (with or without Single Photon Emission Computed Tomography (SPECT)-CT) and pathological assessment (with or without step-serial-sectioning and additional keratin immunohistochemistry (IHC)) [1]. In **chapter 3** we

retrospectively analysed the sensitivity and NPV of the SLNB in a well-defined cohort using watchful waiting as reference standard for the SLNB negative neck and SPECT-CT scanning and step-serial-sectioning with additional keratin staining were part of the protocol. We confirmed the high sensitivity (85%) and NPV (94%) for the detection of occult metastasis in early stage OSCC [11]. Randomized studies which compare the accuracy of the SLNB procedure and END procedures for detecting occult metastasis in OSCC patients with a clinically negative neck (cN0) are not available yet [12]. However, the reported regional recurrence rate in the pN0 staged neck is 6% in a meta-analysis of the SLNB procedure [1] and comparable to the regional recurrence rate in END studies which is reported between 3% to 10% [12-14]. Thus, the SLNB procedure has an accuracy in the detection of occult metastasis in early stage OSCC at least as high as the END procedure with a lower complication and postoperative morbidity rate.

In 2018, a conference about the SLNB in head and neck cancer was held in London with the aim to reach consensus about the SLNB procedure [15,16]. After that conference, consensus about imaging protocols [15] and surgical procedures were [16] reported and a report about the pathological consensus is expected soon. Neck staging with the SLNB can be used in patients with a clinically negative neck based on preoperative imaging using Computed Tomography (CT), Magnetic Resonance Imaging (MRI) or ultrasound with or without fine needle cytology aspiration (USgFNAC) [15,16]. In a review the sensitivity and specificity of these imaging modalities for the detection of occult metastasis in cN0 patients was reported (reviewed in [17]). Although five studies reported high specificities (92- 100%) for CT, US and USgFNAC, these modalities lack sufficiently high sensitivities in cN0 OSCC patients: CT 49-60%, MRI 55-65%, US 48-66% and USgFNAC 73% [18-21] and should therefore always be followed by a SLNB procedure.

Consensus about the preoperative SLNB imaging protocol consisted of two to four mucosal injections peritumourally of the tracer (^{99m}TC-labelled nanocolloids). The injection activity depends on the one (40-50 MBq) or a two day (70-120 MBq) protocol and should be diluted in a maximum volume of 0.4 to 0.5 mL [15]. The injection is immediately followed by dynamic (0-10 min) lymphoscintigraphy and later on by early static (15 min) and late static (2 hrs) lymphoscintigraphy [15]. The lymphoscintigraphy should be followed by SPECT-CT (>2 hrs) [15]. The use of both the SPECT-CT and the lymphoscintigraphy resulted in the additional detection of SLNs in 22% of the cases in a study with 66 SLNB patients [22]. Moreover, in 8% of the cases were non-sentinel lymph nodes reported as SLNs by using planar lymphoscintigraphy only. A hand-held gamma probe is recommended for the intra-operative detection [15,23].

The recommendations in the surgical consensus guidelines include the imaging modalities for the clinical neck staging (at least CTI, MRI or US), sentinel lymph node definition, optical tracers, lymphatic drainage patterns, tumour infiltration depth, size of a positive SLN and follow-up (frequently examination in the first two year) [16]. During surgery, the sentinel lymph nodes are defined as the lymph nodes with an at least 10 times higher count compared to the background and at least a 10% count of the hottest harvested lymph node measured using a hand-held gamma probe [16,23]. The gamma count is not associated with metastasis, or in other words, the metastasis is not always located in the lymph node with the highest count [16]. In general, 2-3 SLNs per patient are detected and removed [16,23-25].

Although the pathological procedure consensus is not reported yet, many centres use step-serial-sectioning and additional IHC nowadays [17,26], after this method was already successfully implemented in other cancer types such as breast, melanoma and endometrium cancer [27-29]. For example, in breast cancer it was reported that up to 40% of the metastases were missed with the conventional single level haematoxylin and eosin (HE) staining [28]. In OSCC, a study showed detection of metastases in 16% of 80 SLNs of OSCC patients using routine HE staining, 23% with the addition of step-serial-sectioning and in 25% with both step-serial-sectioning and additional cytokeratin immunohistochemistry [30]. Especially, isolated tumour cells (defined as metastasis <0.2 mm) were only found with the addition of step-serial-sectioning [30]. In that study the two metastases with the lowest number of isolated tumour cells were only found by using combined step-serial-sectioning and keratin staining. The SLNB protocol used in **chapters 3, 4** and **5** met the recommendations of the consensus meeting about preoperative imaging (cN0 by CT, MRI or USgFNAC and SLN detection by lymphoscintigraphy and SPECT-CT), intraoperative detection using a hand-held gamma probe, postoperative pathological assessment (step-serial-sectioning and additional IHC) and clinical follow-up as reference standard for negative SLNs. Moreover, a median of 3 SLNs per patient was reported comparable with the number in the consensus guidelines. Therefore, the results in **chapter 3** are in accordance with current evidence and recommended guidelines [11].

As described, the SLNB procedure is superior to the END regarding the extend of surgery, morbidity rate and complication rate [7,9,10]. However, also the cost-effectiveness of the SLNB compared to an END or watchful waiting strategy is important. Two studies reported a cost-effectiveness analysis of the SLNB procedure [31,32]. These analyses were based on measurements of costs and quality of life related to early stage OSCC combined with follow-up data of regional recurrences and mortality. Although in one study the SLNB baseline costs were similar compared to the END (€9180,- versus €9241,-) [32], both studies reported that the SLNB procedure was more effective represented by a small increase in quality-adjusted

life years (QALYs) after five years, respectively 3.63 versus 3.61 [32] and 3.70 versus 3.67 [31], equivalent with ~1 week profit in full health. Combining costs and effectiveness resulted in SLNB as most cost-effective strategy for neck staging in early stage OSCC regarding current known sensitivities and NPVs in both studies compared to the END or watchful waiting [31,32]. The fact that initial costs for a less invasive staging technique (SLNB) are comparably to an END procedure [32] could be explained by the additional use of hospital resources: imaging with lymphoscintigraphy and SPECT-CT, additional IHC and second surgery for the neck dissection in case of a positive SLN.

The high accuracy, the cost-effectiveness and the minimally invasive character with low morbidity rates provided an expanding interest and implementation of the SLNB procedure in several national guidelines worldwide as reported in 2017 [33]. However, not all head and neck oncology centres in these countries use SLNB as standard care for the neck staging in early stage OSCC [8,33]. For those units that are interested in the SLNB procedure, it is important to know that success depends on the experience of the surgeon [34]. Inexperienced surgeons can drop the NPV with 5% [35]. Moreover, a dramatically lower sensitivity can be expected with a surgical experience of less than 5 SLNB procedures, while an increase to a 94% sensitivity was reported with an experience of more than 10 SLNB procedures [34,36]. To reduce the risk of a low accuracy in the first period after implementation, Schilling et al. reported a step-wise training program for the implementation of the SLNB procedure [33]. Besides extensive training before the start of the implementation, also a combination of both the SLNB and END is recommended in the first 10 cases to evaluate the obtained accuracy of the SLNB and to prevent patients for regional recurrences as a result of the low experience [33]. In **chapter 3**, we repeated the accuracy analysis after exclusion of the first patients with SLNB neck staging in our centre. The exclusion of these patients resulted not in a lower sensitivity or NPV in our study what might indicate that the SLNB procedure was implemented after thorough training of the surgeons [11]. Also important to notice is the multidisciplinary approach of the SLNB procedure [33]. Not only head and neck surgeons, also the physicians of the nuclear medicine and molecular imaging department must be trained. Moreover regarding the multidisciplinary character, currently, a single-day protocol (tracer dose of 40-50 MBq) or a two day protocol (tracer dose of 70-120 MBq) are recommended options for the SLNB procedure [15]. Single and two-day refer to the day of imaging and surgery. These single or two day protocols are only effective if imaging, surgery and pathology departments work together in a close cooperation and schedules and resources are aligned to each other as was stated in a 10-year evaluation of a study to the SLNB procedure in melanoma [37].

Summarizing, the SLNB procedure used in **chapters 3, 4** and **5** met the recommendations of the current consensus guidelines for SLNB in early stage OSCC. Therefore, the reported

accuracy in detecting occult metastasis in primary early stage OSCC (**chapter 3**) using the SLNB procedure could be used as reference for other centres. The protocol reported in **chapter 3** is a minor surgery alternative for neck staging with the conventional END regarding complications, postoperative morbidity and cost-effectiveness.

Individual lymphatic drainage pattern assessment in head and neck cancer using the SLNB procedure

Another advantage of the SLNB procedure is the assessment of individual lymphatic drainage patterns. Normally in lateralized OSCC tumours, lymphatic drainage goes to lymph nodes located in the ipsilateral levels I-III [38]. An analysis of 583 OSCC patients with T1-T4 staged tumours, revealed no skip metastasis to level IV or V, or in other words, metastases in level IV or V were always accompanied by at least one metastasis in level I-III [39]. In **chapter 3** [11], we did not find skip metastases at the ipsilateral side, however we reported bilateral drainage patterns in 37% of the early stage OSCC cases while also cases with well lateralized tongue tumours were part of this cohort. Moreover, one patient with a well lateralized tongue tumour only had a SLN (negative for metastasis) at the contralateral side of the neck compared to the tumour [11]. Contralateral drainage of lateralized tumours is not uncommon and reported in 10% of the OSCC cases [16,24]. After an END, up to 39% of the regional recurrences are reported in the contralateral neck [40]. The assessment of individual drainage patterns with the detection and harvesting of SLNs with unexpected drainage patterns using the SLNB procedure, might prevent patients for undertreatment. Therefore, if surgical removable, the consensus guidelines recommend harvesting of SLNs located in unexpected locations (i.e. not in ipsilateral levels I-III), because these SLNs might represent an anatomical variation with a direct lymphatic drainage pattern and are therefore potential locations for the first metastasis deposits [16].

The assessment of individual drainage patterns using the SLNB procedure was also helpful in exploring the drainage patterns of patients with a previously treated neck (**chapter 4**) [41,42]. Local or local-regional recurrences are reported in 10-30% of head and neck squamous cell carcinomas (HNSCC) [43]. At the time of diagnosis of a local recurrence or second primary OSCC, many of these patients already underwent a SLNB procedure, neck dissection or radiotherapy for their first primary tumour. Knowledge about how these treatment altered lymphatic drainage patterns was restricted to two studies with a total of 27 patients [44,45]. Therefore, no consensus for the neck strategy of these patients was available in literature. In **chapter 4** [42], we reported on the accuracy of the SLNB in 53 early stage (cT1-2N0) OSCC patients with earlier treatment (dissection or radiotherapy) or surgery (SLNB) of the neck and analysed the lymphatic drainage patterns of these patients. With the low number of events (lymph node metastases 3/45 and regional recurrences 1/45) taken into account, the SLNB procedure seemed accurate in detecting occult metastasis. No drainage patterns

were found for five (12%) patients with a history of radiotherapy. Unexpected drainage (no drainage to ipsilateral levels I-III) was found in 30% of the cases to ipsilateral levels IV and V or to contralateral levels. The altered drainage patterns as a result of the previous neck treatment limit neck staging in cN0 patients with a neck history: which levels needs an END or clinical examination in case of a watchful waiting strategy? Moreover, extensive surgery (neck dissection) in an earlier treated neck is unfavourable because the previous treatment most likely induced fibrosis in the neck. For that reason, the SLNB procedure is currently the most optimal technique for neck staging in previously treated early stage OSCC patients with a clinically negative neck and recommended in the surgical guidelines of the SLNB consensus meeting in 2018 [16].

For a long time, lymphatic drainage of maxillary tumours was thought to be to the para- or retropharyngeal located lymph nodes [46]. Moreover, a lower incidence of lymph node metastases compared to other oral cavity tumours was assumed [46]. In **chapter 5** [41], we have shown that SLNs of patients with maxillary tumours are mainly located in cervical neck levels I-III. However, in 2 out of 10 patients drainage patterns to parapharyngeally located lymph nodes were also detected. In 2016, an overview of the literature on the incidence of lymph node involvement in maxillary tumours from patients with an END neck staging or with a regional recurrence was published [47]. In eight studies reported from 2001 till 2013, an incidence rate of 14% to 38% including all stages of maxillary OSCC was reported [47], which is not lower than that of other oral cavity locations, as was assumed before [46]. Another study reported a similar incidence (14%) but mentioned especially the high rate (46%) of contralateral metastases in cN0 maxillary OSCC patients [48]. Remarkably, none of these ten studies [48-57] on metastases of maxillary OSCC, mentioned lymph node involvement of para- or retropharyngeally located lymph nodes. A review from 2019 analysed the involvement of retropharyngeally located lymph nodes in head and neck cancer [58]. Of all 32 included studies, **chapter 5** [41] was the only included study that used the SLNB procedure to analyse lymphatic drainage patterns to retropharyngeally located lymph nodes in head and neck cancer [58]. Four of the 32 studies [59-61] reported about retropharyngeal lymph node involvement in OSCC. Incidence rates of 1% [60] and 7% [59] were reported for retropharyngeal metastases, which were most seen in, but were not restricted to maxillary OSCC. One study analysed the two-year disease specific (DSS) and diseases free (DFS) survival and reported a dramatically lower survival for patients diagnosed with retropharyngeal metastases during follow-up compared to patients diagnosed with retropharyngeal metastases at initial treatment, respectively 20% versus 13% for DSS and 24% versus 10% for DFS [60].

Taken together, this current knowledge of maxillary OSCC (incidence, bilateral drainage, retropharyngeal drainage, impact on survival and the assessment of individual drainage

patterns with the SLNB procedure as reported in **chapter 5**), the SLNB might also be a suitable neck staging technique in cN0 maxillary OSCC and might prevent these patients for undertreatment of retropharyngeal and contralateral located lymph nodes.

Limitations of the SLNB procedure in oral squamous cell carcinoma

Although the high accuracy in detecting occult metastasis and the assessment of individual lymphatic drainage patterns, the SLNB also has several limitations and uncertainties such as: lower accuracy in floor of mouth tumours, a second surgery in SLNB positive patients and the strategy after a positive SLN with isolated tumour cells.

Higher false negative (regional recurrences) rates up to 25% have been reported for the SLNB procedure in floor of mouth (FOM) tumours [16,62] compared to the 6% in the other OSCCs [1]. This lower accuracy is caused by the shine-through phenomenon [11,63]: the hotspot of the SLN is located within the hotspot of the tumour on lymphoscintigraphy and SPECT-CT. As described also in **chapter 3**, a level I dissection was proposed in a study in which 50% of the SLNs in level I of FOM tumours were detected only intra-operatively using the level I dissection and not preoperatively by lymphoscintigraphy or SPECT-CT [63]. Another option is the combination of a conventional radio-guided tracer with a fluorescence tracer indocyanine green (ICG)-(99m)Tc-nanocolloid [64]. Seventy-five percent of the SLNs in five patients with a FOM tumour were solely detected by this hybrid tracer. The surgical consensus guidelines implemented these options for cases with a high potential of shine-through in level I and recommend a low threshold to explore level I [16].

The clinical value of isolated tumour cells (ITCs) and micrometases, defined as a size of < 0.2 mm and 0.2-2 mm [65,66], respectively, in SLNs of OSCC patients is not completely clear. Before a neoplastic cell has disseminated from the primary tumour site and formed a metastasis in a lymph node (or other organ), these tumour cells have to go through different processes (e.g. detachment, invasion, migration, extravasation, cell division, etc.) [67,68]. If one of these processes is not completely fulfilled, these disseminated tumour cells will most likely not result in a metastasis [67]. For example, a cell can reach a lymph node or other organ, but stay there in a dormant state (disseminated tumour cell (DTC) [67,69,70]. Probably, these DTCs are a result of mechanical manipulation during biopsy [67,71] and lack ability to grow into an invading metastasis [67]. ITCs and the micrometastases detected in lymph nodes by the SLNB procedure might be such dormant DTCs [70] and harvesting of these SLNs might be therapeutic and consequently, these patients might not benefit from an additional MRND. In breast cancer, a recurrence rate of 0.4% was reported in cases with micrometastases in their SLNs (ITC information was not available) with five years of follow-up and without a benefit for patients additionally treated with axillary lymph node dissection [72]. This observation resulted for breast cancer in a pN0(i+) classification for SLNs

with a metastasis size <0.2 mm in the 8th AJCC TNM classification [73]. As a result, in the revised Dutch guideline (in 2017 and 2018) for breast cancer adjuvant axillary treatment (dissection or radiotherapy) is not recommended any more for pN0(i+) SLNs [74]. In **chapter 3** [11], 37% of the SLN metastases of OSCC patients had a metastasis <0.2 mm and none of these patients had additional metastases in their neck dissection specimen, hypothesizing that these SLN positive patients might not need a neck dissection at all or only a selective neck dissection, because of the SLN was the only lymph node harbouring metastases. A low rate of additional lymph nodes in the dissection specimen could also be the reason that postoperative pathological assessment with step-serial-sectioning and additional keratin IHC in addition to the conventional HE staining improved the NPV with only 2% (from 94 to 96%) in a prospective trial [35]. In 2017, a study reported about 234 early stage OSCC patients with positive SLNs and reported metastases sizes from 12 other studies [75]. Additional metastases in the non-SLNs from the neck dissection specimen were found in 13% of the patients with ITCs in their SLNs, 20% of the micrometastases and 40% of the macrometastases (>2 mm) [75]. Although metastasis size in the SLNB of OSCC patients might be associated with the presence of metastasis in non-SLNs in the neck dissection specimen, even the incidence of non-SLN metastasis after ITCs in OSCC (13%) is higher compared to the recurrence rate after ITCs and micrometastases in SLNs of breast cancer patients without a lymph node dissection (0.4%) [75,76]. This low recurrence rate in breast cancer could be explained by the adjuvant systemic therapy which is common in breast cancer treatment: nearly all (~96.5%) patients received systemic therapy (chemo- or hormonal therapy) in the trial that compared the SLNB procedure with or without axillary dissection in breast cancer [75,76]. For that reason, the 2018 consensus guideline for SLNB in OSCC patients recommended to consider SLNs with ITCs as positive SLNs and they needs to be followed by a neck dissection [16]. Probably that characterization of genetic and expression profiles of tumour cells of the primary tumor site and of ITCs in lymph nodes might help to select ITCs with or without metastatic potential [67] and contribute to an even more individualised strategy of the cN0 neck in the future.

Summarising, the SLNB procedure has a lower accuracy in OSCC of the FOM as a result of the shine-through phenomenon (**chapter 3**). Therefore the SLNB procedure must be combined with a level I dissection in FOM tumours. Although we reported no additional metastases in cases with an ITC or micrometastases in their SLN, because of the additional metastases rate in other studies, a MRND after a positive SLN with ITCs or a micrometastasis is still recommended.

Neck staging in OSCC using molecular biomarkers: cortactin as important driver of metastasis

As described more extensively in **chapter 1,** many cellular processes, such as cell motility, determine the metastatic potential of a tumour cell [68,77]. Epigenetic or genetic alterations of genes with a key-role in cell migration are reported as prognostic for lymph node status [68,78]. A review and meta-analysis from 2015 analysed 11q13 chromosome amplification as promising molecular tumour marker for lymph node status in OSCC and found that *CCND1* (one of the genes of that amplicon) and overexpression of its corresponding protein cyclin D1 was a prognostic marker [79]. In **chapter 6** [80] we analysed the clinical value of *CCND1* amplification and the overexpression of three proteins from three major oncogenes (*CTTN*/ cortactin, *CCND1*/cyclin D1 and FADD) located at the 11q13 amplicon in a well-defined cohort of early stage tongue and FOM OSCC with neck staging using the END procedure. All these biomarkers showed promising NPVs for the detection of occult metastasis: *CCND1* copy number 83% and expression of cyclin D1 84%, FADD 81% and cortactin 80% [80]. In **chapter 7** we found that cortactin was predictive for SLN status in small and superficial tumours (7^{Th} TNM pT1 and a tumour infiltration depth <4 mm) with a 92% NPV of early stage OSCC patients with neck staging using the SLNB procedure.

Cortactin is the protein encoded by the *CTTN* gene (also known as *EMS1*), which is one of the genes located at the 11q13 chromosome [79,81,82]. 11q13 amplification is detected in 46% (range 13-100%) of the OSCC cases [82] and *CTTN* is a candidate driver for this amplification [81]. Overexpression of cortactin promotes cell migration and lymph node metastasis in OSCC [82] and is therefore a promising molecular marker to predict lymph node status. Many binding sites have been reported for cortactin by which important cellular processes involved in metastasis are regulated [82], such as binding to Arp2/3 complex in cytoplasm and cell periphery to regulate nucleation and stabilization of actin [83], binding to F-actin in cytoplasm and nucleus to regulate cell migration [84], binding to ZO-1 in cell tight junctions affects the connection between cell-cell adhesion and actin cytoskeleton [85].

One of the best studied cellular processes involved in metastasis in which cortactin plays a key-role is the regulation of protrusive structures [86]. Invadopodia and lamellipodia are these protrusive structures and enable the invasion and migration of a tumour cell by regulating the actin cytoskeleton and extracellular matrix (Figure 1).

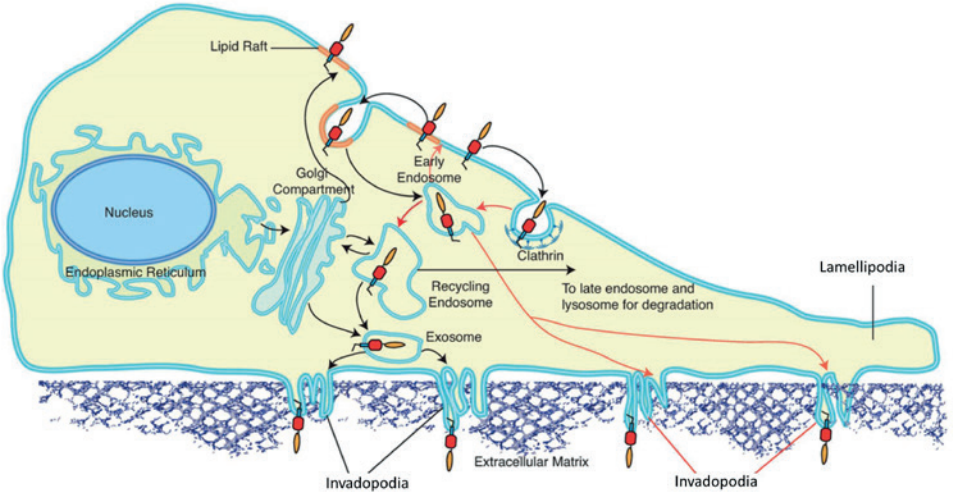

Figure 1. Schematic overview of protrusive structures of a tumour cell mediated by cortactin. This figure shows the protrusive structures invadopodia at the bottom layer and lammellipodia at the front of a tumour cell. This figure shows the delivery to the invadopodia of the membrane type 1 matrix metalloprotease (MT1-MMP, depicted by the green/red/yellow complex) which exposure results in degradation of the extracellular matrix and is one of the cellular processes involved in cell motility regulated by cortactin

[Adapted from Frittoli et al. [87], with permission].

Invadopodia are especially facilitating cell invasion. Formation of invadopodia is reported in a four-stage model by Artym et al. [88] and in the review of Ramos-Garcia et al [82]. During stage I, the invadopia initiation site is characterized by accumulation of cortactin which aggregates with F-actin. During stage II (preinvadopodia), there is more accumulation of the cortactin-F-actin aggregate, resulting in recruitment of membrane type 1 metalloprotease (MT1-MMP). MT1-MMP is an important protein in cell migration by breaking down the extracellular matrix (stage III; mature invadopia) (Figure 1). Cortactin overexpression in stage III promotes also excretion of MT2 and MT3-MMPs into the extracellular matrix and that result in more breakdown [89]. Stage IV (late invadopia) is characterized by the dissolution of cortactin and F-actin while high concentrations of MT1-MMP remain. Lamellipodia are essential for locomotion of cells with an important role for cortactin [82,90]. Protrusion of a lamellipodium starts with actin filament polymerization and elongation which is mediated by cortactin, Arp2/3 complex and the cofilin protein before the other stages of lamellipodium-regulated cell migration take place. In vivo and in vitro studies showed that the expression level of cortactin is related to the cytoplasmic location in squamous cell carcinoma cell lines [82,91,92]. Overexpression of cortactin induces accumulation of cortactin in the cytoskeleton periphery which is essential for the regulation of the protrusive

structures (invadopodia and lamellipodia). Moreover, in vitro studies with squamous cell carcinoma cell lines showed an increase in cell migration with cortactin overexpression [91,93].

A systematic review and meta-analysis from 2019 analysed the clinico-pathological significance of *CTTN*/cortactin alterations in HNSCC [94]. Nine studies (including **chapter 6**) with a low heterogeneity were included for the association with lymph node status in OSCC. Pooled data of these nine studies resulted in a OR 2.78 (95% CI 1.68-4.60) for positive lymph nodes in patients with *CTTN*/cortactin alterations. In that meta-analysis, the authors stated that *CTTN*/cortactin alterations might be helpful to select patients for watchful waiting instead of another neck strategy [94]. However, they recommended further validation of cortactin overexpression with immunohistochemistry (IHC) as preferred approach instead of *CTTN* amplification detection because its simplicity, low cost and routine automatized application in a pathology setting. Moreover, none of nine included studies used data of patients with neck staging using the SLNB procedure. In line with these studies, in **chapter 7** we reported that cortactin overexpression analysed by IHC was associated with SLN status in pT1cN0 OSCCs with a tumour infiltration depth <4 mm.

Morand et al. reported that patients might be selected for their neck strategy preoperatively using tumour infiltration depth [95]. They proposed a model for neck staging wherein patients with a tumour infiltration depth <2 mm receive watchful waiting, 2-5 mm a SLNB and >5 mm an END during first surgery. Leusink et al. proposed a model wherein patients are selected for a watchful waiting strategy instead of a SLNB procedure by a molecular tumour profiling using tumour biopsy specimens [96]. In **chapter 7** we propose that patients with a pT1 staged tumour and a tumour infiltration depth <4 mm and without cortactin overexpression might be selected for a watchful waiting strategy of the neck, while patients with cortactin overexpression or a higher tumour infiltration depth are selected for a SLNB procedure. Larger and prospective studies using tumour biopsy specimens are needed to define and validated the tumour infiltration depth cut-offs for the combination with cortactin.

FUTURE PERSPECTIVES

Detection of occult metastasis

The SLNB procedure is a major step forward in neck staging of early stage OSCC patients compared to the END procedure. However, as long as patients are staged using an invasive procedure and false negatives occur, neck staging needs improvement to reach a more individually successful treatment of early stage patients.

In **chapter 3**, 46% of the SLN positive patients had macrometastasis (>2 mm) which was the case in 40% of the SLN positive patients in nine other studies [75]. The question is, is it possible to detect occult metastases with a higher sensitivity preoperatively? Recently, a review with promising results was published for detecting small metastases using a Combidex-enhanced MRI (CEM) [97]. The CEM is based on the intravenously administration of a solution of ultra-small (20-50 nm) superparamagnetic iron oxides (USPIO) particles (Combidex). CEM was already reported in the nineties, however Combidex was not available for a long time after the manufacturer withdrew Combidex from the registration process in Europe. Recently, the Radboud University Medical Center obtained all rights and manufactured Combidex again. After Combidex is administered, the USPIOs are picked up by macrophages. These macrophages are accumulated in lymph nodes and as a result normal lymph nodes (metastasis negative) lose MR signal (black) while lymph nodes positive for metastases remain white or have a white spot because the macrophages cannot accumulated at the position of the metastases. The difference between black and white lymph nodes enables to distinct positive and negative lymph nodes with a normal size from each other [97]. Moreover, with the CEM it is also possible to detect (pathological) lymph nodes as small as 2 mm [97,98]. Before the withdrawal of Combidex, a very promising 82% sensitivity and 93% NPV for the detection of lymph node metastasis were reported in 375 patients with prostate cancer [99]. In a study with 28 HNSCC patients the sensitivity for the detection of lymph node metastases increased from 52% to 82% after USPIOs were added to the MRI protocol [100]. Although the current evidence with the CEM is mainly from prostate and bladder cancer, Heesakkers et al. stated that this technique might be useable in other cancers as well. With the knowledge that ~25% of the early stage patients are diagnosed with an occult metastasis of which 40% is more than 2 mm in size, it might be that CEM lowers the occult metastasis rate with 10%.

As mentioned earlier, a hybrid tracer with fluorescence is one of the recommendations in the consensus guidelines to prevent false negatives in FOM tumours [16] as a result of the shine-through phenomenon. Besides that hybrid tracer, several experimental techniques are mentioned in the imaging consensus guidelines such as the use of portable gammacameras, freehand SPECT, opto-nuclear probe for acoustic gamma and virtual augmented reality [15]. These techniques are not validated yet, but might be used in the future. Also the use of another radiolabelled colloid tracer was reported [101]. 99mTc-Tilmanocept (Lymphoseek®) has the theoretically advantage of a rapid injection site clearance and a stable binding within lymph nodes as a result of the small particle size (7 nm) and the binding to macrophages by the CD206 receptors [102]. These rapid clearance and stable lymph node accumulation might prevent false negatives caused by shine-through. A phase III trial with 101 included

patients (T1-4, N0) reported promising sensitivity and NPVs which were at least as high as for 99mTc-nannocolloid [101], however a clear validation with both tracers in one study and only early stage OSCC is not available yet.

Detection of local recurrences

In up to 30% of the OSCC, local recurrences or second primary tumours are detected during follow-up [103,104]. As described in **chapter 1**, early detection of local recurrences or second primary tumours is challenging, but essential to improve prognosis. A promising technique to prevent patients for local recurrences is the intra-operative use of image-guided surgery [105] with fluorescently labelled antibodies [106]. Image-guided surgery technique is based on tumour tissue visualisation using a fluorescence tracer conjugated to an antibody [105]. This conjugated antibody binds to an antigen which is upregulated in cancer tissue, resulting in a higher fluorescence signal of tumour tissue compared to surrounding tissues. Using a multispectral fluorescence camera in the operation theatre, this difference in fluorescence can be visualised and used to define surgical resection margins or to visualize residual tumour tissue after initial surgical resection. Currently, clinical trials reported the feasibility and safety of the fluorescently labelled antibodies Panitumumab-IRDye800CW and Cetuximab-IRDye800CW in HNSCC [106,107]. Further studies are needed to confirm that this technique contributes to clear surgical resection margins to prevent patients for re-resections and lower local recurrence rates. As this method is very useful for guiding surgery, this assay might be to insensitive to detect single cells in clear surgical margins. Clear surgical margins are most often defined as tumour cell free margins of at least 5 mm. However, even in patients with clear surgical resection margins, local recurrences are reported in up to 11% of these cases [108,109]. In these particular cases, additional methods might be investigated.

Various molecular methods are available that are able to detect tumour specific mutations at very low levels in a background of normal cells. E.g. the detection of minimal residual cancer with p53 mutations in surgical margins has been reported with promising sensitivities (75% and 85%) but still missed high specificities (67% and 58%) [110]. In **chapter 8** we selected OSCC specific methylation markers with a genome wide approach. Important advantages of hypermethylation markers are the binary state (hypermethylated or not) and DNA hypermethylation appears more often and earlier compared to mutations [77]. These methylation markers might also be useful to detect minimal residual cancer in surgical resection margins that are microscopically tumour cell free.

Another option is to use these methylation markers to detect and monitor circulating tumour cells (CTCs) or circulating tumour DNA (ctDNA) in saliva or plasma of OSCC patients after initial treatment. This might be a useful and non-invasive method to monitor treatment

response after radiotherapy or to an early detection of local or regional recurrences [111]. In **chapter 8** we demonstrated the detection of OSCC cells in saliva using methylation markers. Although some small studies showed already promising results with the detection of CTCs or ctDNA of OSCCs in body fluids such as plasma and saliva, clear validation with large and prospective data is lacking [111].

REFERENCES

1. Liu M, Wang SJ, Yang X, Peng H. Diagnostic Efficacy of Sentinel Lymph Node Biopsy in Early Oral Squamous Cell Carcinoma: A Meta-Analysis of 66 Studies. PLoS One 2017 Jan 20;12(1):e0170322.
2. de Bree R, Takes RP, Shah JP, Hamoir M, Kowalski LP, Robbins KT, et al. Elective neck dissection in oral squamous cell carcinoma: Past, present and future. Oral Oncol 2019 Mar;90:87-93.
3. Weiss MH, Harrison LB, Isaacs RS. Use of decision analysis in planning a management strategy for the stage N0 neck. Arch Otolaryngol Head Neck Surg 1994 Jul;120(7):699-702.
4. Melchers LJ, Schuuring E, van Dijk BA, de Bock GH, Witjes MJ, van der Laan BF, et al. Tumour infiltration depth >/=4 mm is an indication for an elective neck dissection in pT1cN0 oral squamous cell carcinoma. Oral Oncol 2012 Apr;48(4):337-342.
5. Huang SH, Hwang D, Lockwood G, Goldstein DP, O'Sullivan B. Predictive value of tumor thickness for cervical lymph-node involvement in squamous cell carcinoma of the oral cavity: a meta-analysis of reported studies. Cancer 2009 Apr 1;115(7):1489-1497.
6. D'Cruz AK, Vaish R, Kapre N, Dandekar M, Gupta S, Hawaldar R, et al. Elective versus Therapeutic Neck Dissection in Node-Negative Oral Cancer. N Engl J Med 2015 Aug 6;373(6):521-529.
7. Murer K, Huber GF, Haile SR, Stoeckli SJ. Comparison of morbidity between sentinel node biopsy and elective neck dissection for treatment of the n0 neck in patients with oral squamous cell carcinoma. Head Neck 2011 Sep;33(9):1260-1264.
8. Cramer JD, Sridharan S, Ferris RL, Duvvuri U, Samant S. Sentinel Lymph Node Biopsy Versus Elective Neck Dissection for Stage I to II Oral Cavity Cancer. Laryngoscope 2019 Jan;129(1):162-169.
9. Hernando J, Villarreal P, Alvarez-Marcos F, Gallego L, Garcia-Consuegra L, Junquera L. Comparison of related complications: sentinel node biopsy versus elective neck dissection. Int J Oral Maxillofac Surg 2014 Nov;43(11):1307-1312.
10. Schiefke F, Akdemir M, Weber A, Akdemir D, Singer S, Frerich B. Function, postoperative morbidity, and quality of life after cervical sentinel node biopsy and after selective neck dissection. Head Neck 2009 Apr;31(4):503-512.
11. Boeve K, Schepman KP, Schuuring E, Roodenburg JLN, Halmos GB, van Dijk BAC, et al. High sensitivity and negative predictive value of sentinel lymph node biopsy in a retrospective early stage oral cavity cancer cohort in the Northern Netherlands. Clin Otolaryngol 2018 Mar 25.
12. Koyfman SA, Ismaila N, Crook D, D'Cruz A, Rodriguez CP, Sher DJ, et al. Management of the Neck in Squamous Cell Carcinoma of the Oral Cavity and Oropharynx: ASCO Clinical Practice Guideline. J Clin Oncol 2019 Jul 10;37(20):1753-1774.
13. Mizrachi A, Migliacci JC, Montero PH, McBride S, Shah JP, Patel SG, et al. Neck recurrence in clinically node-negative oral cancer: 27-year experience at a single institution. Oral Oncol 2018 Mar;78:94-101.

14. Hosal AS, Carrau RL, Johnson JT, Myers EN. Selective neck dissection in the management of the clinically node-negative neck. Laryngoscope 2000 Dec;110(12):2037-2040.
15. Giammarile F, Schilling C, Gnanasegaran G, Bal C, Oyen WJG, Rubello D, et al. The EANM practical guidelines for sentinel lymph node localisation in oral cavity squamous cell carcinoma. Eur J Nucl Med Mol Imaging 2019 Mar;46(3):623-637.
16. Schilling C, Stoeckli SJ, Vigili MG, de Bree R, Lai SY, Alvarez J, et al. Surgical consensus guidelines on sentinel node biopsy (SNB) in patients with oral cancer. Head Neck 2019 Aug;41(8):2655-2664.
17. de Bree R, Takes RP, Castelijns JA, Medina JE, Stoeckli SJ, Mancuso AA, et al. Advances in diagnostic modalities to detect occult lymph node metastases in head and neck squamous cell carcinoma. Head Neck 2015 Dec;37(12):1829-1839.
18. van den Brekel MW, Castelijns JA, Stel HV, Golding RP, Meyer CJ, Snow GB. Modern imaging techniques and ultrasound-guided aspiration cytology for the assessment of neck node metastases: a prospective comparative study. Eur Arch Otorhinolaryngol 1993;250(1):11-17.
19. Righi PD, Kopecky KK, Caldemeyer KS, Ball VA, Weisberger EC, Radpour S. Comparison of ultrasound-fine needle aspiration and computed tomography in patients undergoing elective neck dissection. Head Neck 1997 Oct;19(7):604-610.
20. Takes RP, Righi P, Meeuwis CA, Manni JJ, Knegt P, Marres HA, et al. The value of ultrasound with ultrasound-guided fine-needle aspiration biopsy compared to computed tomography in the detection of regional metastases in the clinically negative neck. Int J Radiat Oncol Biol Phys 1998 Mar 15;40(5):1027-1032.
21. Liao LJ, Lo WC, Hsu WL, Wang CT, Lai MS. Detection of cervical lymph node metastasis in head and neck cancer patients with clinically N0 neck-a meta-analysis comparing different imaging modalities. BMC Cancer 2012 Jun 12;12:236-2407-12-236.
22. den Toom IJ, van Schie A, van Weert S, Karagozoglu KH, Bloemena E, Hoekstra OS, et al. The added value of SPECT-CT for the identification of sentinel lymph nodes in early stage oral cancer. Eur J Nucl Med Mol Imaging 2017 Jun;44(6):998-1004.
23. Alkureishi LW, Burak Z, Alvarez JA, Ballinger J, Bilde A, Britten AJ, et al. Joint practice guidelines for radionuclide lymphoscintigraphy for sentinel node localization in oral/oropharyngeal squamous cell carcinoma. Eur J Nucl Med Mol Imaging 2009 Nov;36(11):1915-1936.
24. Schilling C, Stoeckli SJ, Haerle SK, Broglie MA, Huber GF, Sorensen JA, et al. Sentinel European Node Trial (SENT): 3-year results of sentinel node biopsy in oral cancer. Eur J Cancer 2015 Dec;51(18):2777-2784.
25. Pedersen NJ, Jensen DH, Hedback N, Frendo M, Kiss K, Lelkaitis G, et al. Staging of early lymph node metastases with the sentinel lymph node technique and predictive factors in T1/T2 oral cavity cancer: A retrospective single-center study. Head Neck 2016 Apr;38 Suppl 1:E1033-40.
26. Den Toom IJ, Heuveling DA, Flach GB, van Weert S, Karagozoglu KH, van Schie A, et al. Sentinel node biopsy for early-stage oral cavity cancer: the VU University

Medical Center experience. Head Neck 2015 Apr;37(4):573-578.
27. Delpech Y, Cortez A, Coutant C, Callard P, Uzan S, Darai E, et al. The sentinel node concept in endometrial cancer: histopathologic validation by serial section and immunohistochemistry. Ann Oncol 2007 Nov;18(11):1799-1803.
28. de Boer M, van Dijck JA, Bult P, Borm GF, Tjan-Heijnen VC. Breast cancer prognosis and occult lymph node metastases, isolated tumor cells, and micrometastases. J Natl Cancer Inst 2010 Mar 17;102(6):410-425.
29. Gietema HA, Vuylsteke RJ, de Jonge IA, van Leeuwen PA, Molenkamp BG, van der Sijp JR, et al. Sentinel lymph node investigation in melanoma: detailed analysis of the yield from step sectioning and immunohistochemistry. J Clin Pathol 2004 Jun;57(6):618-620.
30. Trivedi NP, Ravindran HK, Sundram S, Iyer S, Kekatpure V, Durah S, et al. Pathologic evaluation of sentinel lymph nodes in oral squamous cell carcinoma. Head Neck 2010 Nov;32(11):1437-1443.
31. van der Linden N, Flach GB, de Bree R, Uyl-de Groot CA. Cost-utility of sentinel lymph node biopsy in cT1-T2N0 oral cancer. Oral Oncol 2016 Feb;53:20-26.
32. Govers TM, Takes RP, Baris Karakullukcu M, Hannink G, Merkx MA, Grutters JP, et al. Management of the N0 neck in early stage oral squamous cell cancer: a modeling study of the cost-effectiveness. Oral Oncol 2013 Aug;49(8):771-777.
33. Schilling C, Shaw R, Schache A, McMahon J, Chegini S, Kerawala C, et al. Sentinel lymph node biopsy for oral squamous cell carcinoma. Where are we now? Br J Oral Maxillofac Surg 2017 Oct;55(8):757-762.
34. Bluemel C, Rubello D, Colletti PM, de Bree R, Herrmann K. Sentinel lymph node biopsy in oral and oropharyngeal squamous cell carcinoma: current status and unresolved challenges. Eur J Nucl Med Mol Imaging 2015 Aug;42(9):1469-1480.
35. Civantos FJ, Zitsch RP, Schuller DE, Agrawal A, Smith RB, Nason R, et al. Sentinel lymph node biopsy accurately stages the regional lymph nodes for T1-T2 oral squamous cell carcinomas: results of a prospective multi-institutional trial. J Clin Oncol 2010 Mar 10;28(8):1395-1400.
36. Ross GL, Shoaib T, Soutar DS, MacDonald DG, Camilleri IG, Bessent RG, et al. The First International Conference on Sentinel Node Biopsy in Mucosal Head and Neck Cancer and adoption of a multicenter trial protocol. Ann Surg Oncol 2002 May;9(4):406-410.
37. Bagaria SP, Faries MB, Morton DL. Sentinel node biopsy in melanoma: technical considerations of the procedure as performed at the John Wayne Cancer Institute. J Surg Oncol 2010 Jun 15;101(8):669-676.
38. Guo CB, Feng Z, Zhang JG, Peng X, Cai ZG, Mao C, et al. Supraomohyoid neck dissection and modified radical neck dissection for clinically node-negative oral squamous cell carcinoma: a prospective study of prognosis, complications and quality of life. J Craniomaxillofac Surg 2014 Dec;42(8):1885-1890.
39. Pantvaidya GH, Pal P, Vaidya AD, Pai PS, D'Cruz AK. Prospective study of 583 neck dissections in oral cancers: implications for clinical practice. Head Neck 2014 Oct;36(10):1503-1507.

40. Ganly I, Goldstein D, Carlson DL, Patel SG, O'Sullivan B, Lee N, et al. Long-term regional control and survival in patients with "low-risk," early stage oral tongue cancer managed by partial glossectomy and neck dissection without postoperative radiation: the importance of tumor thickness. Cancer 2013 Mar 15;119(6):1168-1176.
41. Boeve K, Schepman KP, Vegt BV, Schuuring E, Roodenburg JL, Brouwers AH, et al. Lymphatic drainage patterns of oral maxillary tumors: Approachable locations of sentinel lymph nodes mainly at the cervical neck level. Head Neck 2017 Mar;39(3):486-491.
42. den Toom IJ, Boeve K, van Weert S, Bloemena E, Brouwers AH, Hoekstra OS, et al. High rate of unexpected lymphatic drainage patterns and a high accuracy of the sentinel lymph node biopsy in oral cancer after previous neck treatment. Oral Oncol 2019 Jul;94:68-72.
43. Leemans CR, Tiwari R, Nauta JJ, van der Waal I, Snow GB. Regional lymph node involvement and its significance in the development of distant metastases in head and neck carcinoma. Cancer 1993 Jan 15;71(2):452-456.
44. Flach GB, Broglie MA, van Schie A, Bloemena E, Leemans CR, de Bree R, et al. Sentinel node biopsy for oral and oropharyngeal squamous cell carcinoma in the previously treated neck. Oral Oncol 2012 Jan;48(1):85-89.
45. Pitman KT, Johnson JT, Brown ML, Myers EN. Sentinel lymph node biopsy in head and neck squamous cell carcinoma. Laryngoscope 2002 Dec;112(12):2101-2113.
46. Umeda M, Minamikawa T, Yokoo S, Komori T. Metastasis of maxillary carcinoma to the parapharyngeal space: rationale and technique for concomitant en bloc parapharyngeal dissection. J Oral Maxillofac Surg 2002 Apr;60(4):408-13; discussion 413-4.
47. Os AD, Karakullukcu B, Leemans CR, Halmos GB, Roodenburg JL, Weert SV, et al. Management of the clinically N0 neck in squamous cell carcinoma of the maxillary alveolus and hard palate. Head Neck 2016 Dec;38(12):1794-1798.
48. Joosten MHMA, de Bree R, Van Cann EM. Management of the clinically node negative neck in squamous cell carcinoma of the maxilla. Oral Oncol 2017 Mar;66:87-92.
49. van Os AD, Karakullukcu B, Leemans CR, Halmos GB, Roodenburg JL, van Weert S, et al. Management of the clinically N0 neck in squamous cell carcinoma of the maxillary alveolus and hard palate. Head Neck 2016 Jul 4.
50. Montes DM, Schmidt BL. Oral maxillary squamous cell carcinoma: management of the clinically negative neck. J Oral Maxillofac Surg 2008 Apr;66(4):762-766.
51. Montes DM, Carlson ER, Fernandes R, Ghali GE, Lubek J, Ord R, et al. Oral maxillary squamous carcinoma: an indication for neck dissection in the clinically negative neck. Head Neck 2011 Nov;33(11):1581-1585.
52. Yorozu A, Sykes AJ, Slevin NJ. Carcinoma of the hard palate treated with radiotherapy: a retrospective review of 31 cases. Oral Oncol 2001 Sep;37(6):493-497.
53. Simental AA,Jr, Johnson JT, Myers EN. Cervical metastasis from squamous cell carcinoma of the maxillary alveolus

and hard palate. Laryngoscope 2006 Sep;116(9):1682-1684.
54. Lin HW, Bhattacharyya N. Survival impact of nodal disease in hard palate and maxillary alveolus cancer. Laryngoscope 2009 Feb;119(2):312-315.
55. Ogura I, Kurabayashi T, Sasaki T, Amagasa T, Okada N, Kaneda T. Maxillary bone invasion by gingival carcinoma as an indicator of cervical metastasis. Dentomaxillofac Radiol 2003 Sep;32(5):291-294.
56. Poeschl PW, Seemann R, Czembirek C, Russmueller G, Sulzbacher I, Selzer E, et al. Impact of elective neck dissection on regional recurrence and survival in cN0 staged oral maxillary squamous cell carcinoma. Oral Oncol 2012 Feb;48(2):173-178.
57. Brown JS, Bekiroglu F, Shaw RJ, Woolgar JA, Rogers SN. Management of the neck and regional recurrence in squamous cell carcinoma of the maxillary alveolus and hard palate compared with other sites in the oral cavity. Head Neck 2013 Feb;35(2):265-269.
58. Doumas S, Fort-Schaale A, Skoulakis C, Moor J, Kanatas A. Current thinking on the management of abnormal retropharyngeal nodes in patients with oral, oropharyngeal, and nasopharyngeal squamous cell carcinoma: a structured review. Br J Oral Maxillofac Surg 2019 Jul;57(6):515-528.
59. Umeda M, Minamikawa T, Komatsubara H, Ojima Y, Shibuya Y, Yokoo S, et al. En bloc resection of the primary tumour and cervical lymph nodes through the parapharyngeal space in patients with squamous cell carcinoma of the maxilla: a preliminary study. Br J Oral Maxillofac Surg 2005 Feb;43(1):17-22.
60. Tseng JR, Ho TY, Lin CY, Lee LY, Wang HM, Liao CT, et al. Clinical outcomes of patients with oral cavity squamous cell carcinoma and retropharyngeal lymph node metastasis identified by FDG PET/CT. PLoS One 2013 Nov 11;8(11):e79766.
61. Umeda M, Shigeta T, Takahashi H, Kataoka T, Oguni A, Minamikawa T, et al. Metastasis to the lateral retropharyngeal lymph node from squamous cell carcinoma of the oral cavity: report of three cases. Int J Oral Maxillofac Surg 2009 Sep;38(9):1004-1008.
62. Alkureishi LW, Ross GL, Shoaib T, Soutar DS, Robertson AG, Thompson R, et al. Sentinel node biopsy in head and neck squamous cell cancer: 5-year follow-up of a European multicenter trial. Ann Surg Oncol 2010 Sep;17(9):2459-2464.
63. Stoeckli SJ, Huebner T, Huber GF, Broglie MA. Technique for reliable sentinel node biopsy in squamous cell carcinomas of the floor of mouth. Head Neck 2016 Sep;38(9):1367-1372.
64. van den Berg NS, Brouwer OR, Klop WM, Karakullukcu B, Zuur CL, Tan IB, et al. Concomitant radio- and fluorescence-guided sentinel lymph node biopsy in squamous cell carcinoma of the oral cavity using ICG-(99m)Tc-nanocolloid. Eur J Nucl Med Mol Imaging 2012 Jul;39(7):1128-1136.
65. Hermanek P, Hutter RV, Sobin LH, Wittekind C. International Union Against Cancer. Classification of isolated tumor cells and micrometastasis. Cancer 1999 Dec 15;86(12):2668-2673.
66. Ferlito A, Rinaldo A, Devaney KO, Nakashiro K, Hamakawa H. Detection of lymph node micrometastases in patients

with squamous carcinoma of the head and neck. Eur Arch Otorhinolaryngol 2008 Oct;265(10):1147-1153.
67. Dasgupta A, Lim AR, Ghajar CM. Circulating and disseminated tumor cells: harbingers or initiators of metastasis? Mol Oncol 2017 Jan;11(1):40-61.
68. Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. Nat Rev Cancer 2011 Jan;11(1):9-22.
69. Gao XL, Zhang M, Tang YL, Liang XH. Cancer cell dormancy: mechanisms and implications of cancer recurrence and metastasis. Onco Targets Ther 2017 Oct 27;10:5219-5228.
70. Sproll C, Fluegen G, Stoecklein NH. Minimal Residual Disease in Head and Neck Cancer and Esophageal Cancer. Adv Exp Med Biol 2018;1100:55-82.
71. Kaifi JT, Li G, Clawson G, Kimchi ET, Staveley-O'Carroll KF. Perioperative circulating tumor cell detection: Current perspectives. Cancer Biol Ther 2016 Aug 2;17(8):859-869.
72. Bilimoria KY, Bentrem DJ, Hansen NM, Bethke KP, Rademaker AW, Ko CY, et al. Comparison of sentinel lymph node biopsy alone and completion axillary lymph node dissection for node-positive breast cancer. J Clin Oncol 2009 Jun 20;27(18):2946-2953.
73. Cserni G, Chmielik E, Cserni B, Tot T. The new TNM-based staging of breast cancer. Virchows Arch 2018 May;472(5):697-703.
74. Dutch guidelines breast cancer. 2018; Available at: https://richtlijnendatabase.nl/richtlijn/borstkanker/invasief_carcinoom.html. Accessed August 08, 2019.
75. Den Toom IJ, Bloemena E, van Weert S, Karagozoglu KH, Hoekstra OS, de Bree R. Additional non-sentinel lymph node metastases in early oral cancer patients with positive sentinel lymph nodes. Eur Arch Otorhinolaryngol 2017 Feb;274(2):961-968.
76. Giuliano AE, Ballman K, McCall L, Beitsch P, Whitworth PW, Blumencranz P, et al. Locoregional Recurrence After Sentinel Lymph Node Dissection With or Without Axillary Dissection in Patients With Sentinel Lymph Node Metastases: Long-term Follow-up From the American College of Surgeons Oncology Group (Alliance) ACOSOG Z0011 Randomized Trial. Ann Surg 2016 Sep;264(3):413-420.
77. Esteller M. Epigenetics in cancer. N Engl J Med 2008 Mar 13;358(11):1148-1159.
78. van Hooff SR, Leusink FK, Roepman P, Baatenburg de Jong RJ, Speel EJ, van den Brekel MW, et al. Validation of a gene expression signature for assessment of lymph node metastasis in oral squamous cell carcinoma. J Clin Oncol 2012 Nov 20;30(33):4104-4110.
79. Noorlag R, van Kempen PM, Stegeman I, Koole R, van Es RJ, Willems SM. The diagnostic value of 11q13 amplification and protein expression in the detection of nodal metastasis from oral squamous cell carcinoma: a systematic review and meta-analysis. Virchows Arch 2015 Apr;466(4):363-373.
80. Noorlag R, Boeve K, Witjes MJ, Koole R, Peeters TL, Schuuring E, et al. Amplification and protein overexpression of cyclin D1: Predictor of occult nodal metastasis in early oral cancer. Head Neck 2017 Feb;39(2):326-333.
81. Gibcus JH, Menkema L, Mastik MF, Hermsen MA, de Bock GH, van Velthuysen ML, et al. Amplicon mapping and

expression profiling identify the Fas-associated death domain gene as a new driver in the 11q13.3 amplicon in laryngeal/pharyngeal cancer. Clin Cancer Res 2007 Nov 1;13(21):6257-6266.
82. Ramos-Garcia P, Gonzalez-Moles MA, Gonzalez-Ruiz L, Ayen A, Ruiz-Avila I, Navarro-Trivino FJ, et al. An update of knowledge on cortactin as a metastatic driver and potential therapeutic target in oral squamous cell carcinoma. Oral Dis 2019 May;25(4):949-971.
83. Weed SA, Karginov AV, Schafer DA, Weaver AM, Kinley AW, Cooper JA, et al. Cortactin localization to sites of actin assembly in lamellipodia requires interactions with F-actin and the Arp2/3 complex. J Cell Biol 2000 Oct 2;151(1):29-40.
84. Zhang Y, Zhang M, Dong H, Yong S, Li X, Olashaw N, et al. Deacetylation of cortactin by SIRT1 promotes cell migration. Oncogene 2009 Jan 22;28(3):445-460.
85. Katsube T, Togashi S, Hashimoto N, Ogiu T, Tsuji H. Filamentous actin binding ability of cortactin isoforms is responsible for their cell-cell junctional localization in epithelial cells. Arch Biochem Biophys 2004 Jul 1;427(1):79-90.
86. Schuuring E, Verhoeven E, Litvinov S, Michalides RJ. The product of the EMS1 gene, amplified and overexpressed in human carcinomas, is homologous to a v-src substrate and is located in cell-substratum contact sites. Mol Cell Biol 1993 May;13(5):2891-2898.
87. Frittoli E, Palamidessi A, Disanza A, Scita G. Secretory and endo/exocytic trafficking in invadopodia formation: the MT1-MMP paradigm. Eur J Cell Biol 2011 Feb-Mar;90(2-3):108-114.
88. Artym VV, Zhang Y, Seillier-Moiseiwitsch F, Yamada KM, Mueller SC. Dynamic interactions of cortactin and membrane type 1 matrix metalloproteinase at invadopodia: defining the stages of invadopodia formation and function. Cancer Res 2006 Mar 15;66(6):3034-3043.
89. Clark ES, Whigham AS, Yarbrough WG, Weaver AM. Cortactin is an essential regulator of matrix metalloproteinase secretion and extracellular matrix degradation in invadopodia. Cancer Res 2007 May 1;67(9):4227-4235.
90. Yilmaz M, Christofori G. EMT, the cytoskeleton, and cancer cell invasion. Cancer Metastasis Rev 2009 Jun;28(1-2):15-33.
91. van Rossum AG, de Graaf JH, Schuuring-Scholtes E, Kluin PM, Fan YX, Zhan X, et al. Alternative splicing of the actin binding domain of human cortactin affects cell migration. J Biol Chem 2003 Nov 14;278(46):45672-45679.
92. van Damme H, Brok H, Schuuring-Scholtes E, Schuuring E. The redistribution of cortactin into cell-matrix contact sites in human carcinoma cells with 11q13 amplification is associated with both overexpression and post-translational modification. J Biol Chem 1997 Mar 14;272(11):7374-7380.
93. Rothschild BL, Shim AH, Ammer AG, Kelley LC, Irby KB, Head JA, et al. Cortactin overexpression regulates actin-related protein 2/3 complex activity, motility, and invasion in carcinomas with chromosome 11q13 amplification. Cancer Res 2006 Aug 15;66(16):8017-8025.

94. Ramos-Garcia P, Gonzalez-Moles MA, Ayen A, Gonzalez-Ruiz L, Ruiz-Avila I, Gil-Montoya JA. Prognostic and clinicopathological significance of CTTN/cortactin alterations in head and neck squamous cell carcinoma: Systematic review and meta-analysis. Head Neck 2019 Jun;41(6):1963-1978.
95. Morand GB, Ikenberg K, Vital DG, Cardona I, Moch H, Stoeckli SJ, et al. Preoperative assessment of CD44-mediated depth of invasion as predictor of occult metastases in early oral squamous cell carcinoma. Head Neck 2019 Apr;41(4):950-958.
96. Leusink FK, van Es RJ, de Bree R, Baatenburg de Jong RJ, van Hooff SR, Holstege FC, et al. Novel diagnostic modalities for assessment of the clinically node-negative neck in oral squamous-cell carcinoma. Lancet Oncol 2012 Dec;13(12):e554-61.
97. Fortuin AS, Bruggemann R, van der Linden J, Panfilov I, Israel B, Scheenen TWJ, et al. Ultra-small superparamagnetic iron oxides for metastatic lymph node detection: back on the block. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2018 Jan;10(1):10.1002/wnan.1471. Epub 2017 Apr 6.
98. Fortuin AS, Deserno WM, Meijer HJ, Jager GJ, Takahashi S, Debats OA, et al. Value of PET/CT and MR lymphography in treatment of prostate cancer patients with lymph node metastases. Int J Radiat Oncol Biol Phys 2012 Nov 1;84(3):712-718.
99. Heesakkers RA, Hovels AM, Jager GJ, van den Bosch HC, Witjes JA, Raat HP, et al. MRI with a lymph-node-specific contrast agent as an alternative to CT scan and lymph-node dissection in patients with prostate cancer: a prospective multicohort study. Lancet Oncol 2008 Sep;9(9):850-856.
100. Baghi M, Mack MG, Hambek M, Rieger J, Vogl T, Gstoettner W, et al. The efficacy of MRI with ultrasmall superparamagnetic iron oxide particles (USPIO) in head and neck cancers. Anticancer Res 2005 Sep-Oct;25(5):3665-3670.
101. Agrawal A, Civantos FJ, Brumund KT, Chepeha DB, Hall NC, Carroll WR, et al. (99m)Tc]Tilmanocept Accurately Detects Sentinel Lymph Nodes and Predicts Node Pathology Status in Patients with Oral Squamous Cell Carcinoma of the Head and Neck: Results of a Phase III Multi-institutional Trial. Ann Surg Oncol 2015 Oct;22(11):3708-3715.
102. Marcinow AM, Hall N, Byrum E, Teknos TN, Old MO, Agrawal A. Use of a novel receptor-targeted (CD206) radiotracer, 99mTc-tilmanocept, and SPECT/CT for sentinel lymph node detection in oral cavity squamous cell carcinoma: initial institutional report in an ongoing phase 3 study. JAMA Otolaryngol Head Neck Surg 2013 Sep;139(9):895-902.
103. Leemans CR, Tiwari R, Nauta JJ, van der Waal I, Snow GB. Recurrence at the primary site in head and neck cancer and the significance of neck lymph node metastases as a prognostic factor. Cancer 1994 Jan 1;73(1):187-190.
104. Licciardello JT, Spitz MR, Hong WK. Multiple primary cancer in patients with cancer of the head and neck: second cancer of the head and neck, esophagus, and lung. Int J Radiat Oncol Biol Phys 1989 Sep;17(3):467-476.
105. de Boer E, Harlaar NJ, Taruttis A, Nagengast WB, Rosenthal EL, Ntziachristos

V, et al. Optical innovations in surgery. Br J Surg 2015 Jan;102(2):e56-72.
106. Rosenthal EL, Warram JM, de Boer E, Chung TK, Korb ML, Brandwein-Gensler M, et al. Safety and Tumor Specificity of Cetuximab-IRDye800 for Surgical Navigation in Head and Neck Cancer. Clin Cancer Res 2015 Aug 15;21(16):3658-3666.
107. Gao RW, Teraphongphom N, de Boer E, van den Berg NS, Divi V, Kaplan MJ, et al. Safety of panitumumab-IRDye800CW and cetuximab-IRDye800CW for fluorescence-guided surgical navigation in head and neck cancers. Theranostics 2018 Mar 28;8(9):2488-2495.
108. Tasche KK, Buchakjian MR, Pagedar NA, Sperry SM. Definition of "Close Margin" in Oral Cancer Surgery and Association of Margin Distance With Local Recurrence Rate. JAMA Otolaryngol Head Neck Surg 2017 Dec 1;143(12):1166-1172.
109. Nason RW, Binahmed A, Pathak KA, Abdoh AA, Sandor GK. What is the adequate margin of surgical resection in oral cancer? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009 May;107(5):625-629.
110. Braakhuis BJ, Bloemena E, Leemans CR, Brakenhoff RH. Molecular analysis of surgical margins in head and neck cancer: more than a marginal issue. Oral Oncol 2010 Jul;46(7):485-491.
111. Arantes LMRB, De Carvalho AC, Melendez ME, Lopes Carvalho A. Serum, plasma and saliva biomarkers for head and neck cancer. Expert Rev Mol Diagn 2018 Jan;18(1):85-112.